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(54) Stabilization of antibodies.

(57) According to the present invention antibody preparations are more stabile during storage if in addition they contain a mixture of at least one polyoxypropylene-polyoxyethylene block polymer (such as Pluronic F68) and at least one phospholipid (such as lecithin).

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Stabilization of antibodies

This application relates to a stable aqueous solution of antibodies.

To an increasing extent, antibodies are being used in human and in veterinary medicine both for prophylactic and for diagnostic and therapeutic purposes. The antibodies used in this manner are at present primarily monoclonal antibodies which can be obtained with high purity from a culture of immortalized B-lymphocytes.

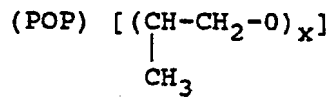
Important fields of application for antibodies are, inter alia, the prevention or cure of infectious diseases (for which, for example, antiviral, antibacterial or antiparasitic antibodies are administered) the regulation of hormone levels (in particular, of gonadotropins, for which anti-gonadotropins are administered) and the localization and/or combating of tumours (for which antibodies, optionally bonded to a labelling substance or therapeutic agent, directed against specific tumour antigens are administered).

In all these applications, the problem is to keep the aqueous solution of the antibodies stable for a sufficiently long time, not only with respect to the activity but, in particular, also with respect to the physical state of the antibody molecules. This physical instability of antibody solutions often results in aggregate formation and, in the long term, in sedimentation of the antibodies. As a result of this, constant quality of the product cannot be guaranteed, which is unacceptable for pharmaceutical products.

It has now been found that aqueous solutions of antibodies are physically stable for a sufficiently long time if they also contain a combination of a polyoxypropylene-polyoxyethylene block polymer (POP-POE block polymer) and a phospholipid.

No aggregation of the antibodies occurs in such a composition so that the solution remains clear and homogeneous while the activity of the peptide also remains intact.

The POP-POE block polymers, also termed poloxamers, are marketed under trade names such as Pluronic(R), Synperonic(R), Supronic(R) and Emkalyx(R). These are compounds which consist of blocks of polyoxypropylene



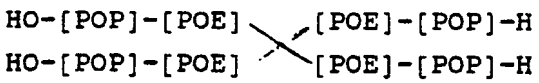
and polyoxyethylene (POE) $[(CH_2\text{-}CH_2\text{-}O)_y]$ (wherein x and y are integers) which respectively form the hydrophobic and hydrophilic components of such block polymers. Known block polymer compounds are of the normal three-block type:

HO-[POE]-[POP]-[POE]-H

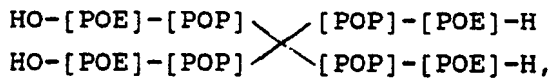
which include the Pluronics L31, L81, L92, L101, L131, L122, P103, F68 and F108, or of the reverse three-block type:

HO-[POP]-[POE]-[POP]-H

which include the Pluronics 25R1 and 31R1, or of the normal eight-block type:



which include the Pluronics T1101, T1301 and T1501, or of the reverse eight-block type:



represented by, inter alia, the Pluronics T90R1, T110R1, T130R1, T130R2, T150R1, T150R4 and T150R8.

The difference between these four types stems from the differences in average chain length of the respective POP and POE blocks.

In general, use is preferably made of POP-POE three-block polymers having a mean molecular weight between approximately 950 and 4,000 and having a polyoxy-ethylene content of up to approximately 80%.

Within the scope of the present invention, the most suitable representatives have, in view of their water solubility, a POE content of greater than 50%, such as, for example, Pluronic F68.

The phospholipids are esters of phosphoric acid and occur, inter alia, in lecithin. The quantitative composition of lecithin varies depending on the source. The phospholipids in lecithin comprise tens of compounds, of which the most important are phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol. In addition, phosphatidyl serine, diphosphatidyl glycerol, sphingomyelin, phosphatidic acid and lysophospholipids. These components can be obtained in more or less pure form from lecithin, but if desired, they can also be prepared synthetically. According to the present invention, the aqueous antibody solution may contain, for example, lecithin, or a fraction thereof, or a component thereof or a mixture of two or more of these components. Advantageously, use can be made of a mixture which consists mainly of phosphatidyl choline and phosphatidyl ethanolamine (preferably, at least approximately 90%) and small quantities of phosphatidyl inositol and lysophospholipids.

The quantity of block polymer in the solution according to the invention is preferably between 0.01 and 5%, the quantity of phospholipids preferably between 0.0001 and 1%, and the concentration of antibodies is preferably between 0.001 and 1 mg/ml.

The antibodies which can be stabilized according to the present invention may, for example, consist of, or be obtained from, antiserum (polyclonal antibodies) or be produced by immortalized B-lymphocytes (monoclonal antibodies), or possibly by triomas or quadromas (which produce bivalent monoclonal antibodies) or by preferably eukaryotic host cells which have been transformed with recombinant DNA, at least a part of which codes for a (possibly chimaeric) antibody or an antigen-bonding fragment thereof.

The relevant antibodies may be directed against any antigen or hapten of, for example, diagnostic, prognostic, therapeutic or prophylactic importance. Suitable antigens are, for example, directed against hormones and, in particular, against gonadotropic hormones such as human chorionic gonadotropin, follicle-stimulating hormone, lutenizing hormone, "pregnant mare serum gonadotropin" (PMSG), and human menopausal gonadotropin.

In livestock breeding, PMSG is used to promote pregnancy, and specifically, the number of offspring. Antibodies for PMSG (anti-PMSG) are then administered after some time to eliminate the disadvantageous effects of a high PMSG content in the blood for the fertilized egg cell.

The invention is explained by reference to the following examples.

Example 1

Monoclonal anti-PMSG	(R)	880	µg
Phospholipid mixture (Infusol)		10	µg
Pluronic F68		3	mg
Glycine		7.06	mg
Benzyl alcohol		10	mg
Water for injection to make		1	ml

Example 2

Monoclonal anti-PMSG	(R)	200	µg
Phospholipid mixture (Infusol)		10	µg
Pluronic F68		6	mg
Glycine		7.06	mg
Benzyl alcohol		10	mg
Water for injection to make		1	ml

Example 3

Monoclonal anti-HCG		0.75	mg
Phospholipon 100		100	µg
Pluronic F87		1	mg
Phosphate buffer, 0.07 M, pH = 8		0.9	ml
Water for injection to make		1	ml

Example 4

Monoclonal anti-K99		0.85	mg
Phospholipon 100		100	µg
Pluronic F38		1	mg
Thiomersal		0.1	mg
Glucose		100	mg
Phosphate buffer, 0.05 M, pH = 7		0.8	ml
Purified water to make		1	ml

Example 5

Monoclonal anti-PST (porcine somatotropin)		3.0	mg
Epicuron 125		0.5	mg
Pluronic L121		10	mg
Carbonate buffer 0.05M pH = 8.5		0.6	ml
Water for injection	ad	1	ml

Example 6

Monoclonal anti-GnRH		2.5	mg
Infusol		50	µg
Pluronic F38		1	mg
Glycocol		7.5	mg
Benzylalcohol		10	mg
Water for injection	ad	1	ml

Example 7

Monoclonal anti-inhibin		1.0	mg
Lecithin		10	mg
Pluronic L121		50	mg
Phosphate buffer pH = 6		0.6	ml
Methylparaten		1.0	mg
Water for injection	ad	1	ml

The stability and activity of the antibody solutions described in Examples 1 and 2 have been studied for many months. At the same time, they were compared with solutions which contained no POP-POE block polymer and phospholipid. The results are shown in the table below:

Preparation No.	7900 Monoclonal 167 µg/ml; benzyl alcohol; 1% glycine buffer			8285 Monoclonal 167 µg/ml; gelatin A 0.1%; benzyl alcohol 1%; glycine buffer			8488 (Example 1) Monoclonal 880 µg/ml; Infusol 0.001%; Pluronic F68 0.3%; benzyl alcohol 1%; glycine buffer			8984 (Example 2) Monoclonal 200 µg/ml; Infusol0.001 %;Pluronic F68 0.6%; benzyl alcohol 1%; glycine buffer			9566 Monoclonal 200 µg/ml; Infusol 0.001%;Pluronic F68 0.3%; benzyl alcohol 1%; glycine buffer		
Physical stability	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C	4°C	25°C	37°C
T_0	+			+			+			+			+		
1 week	+	+	-	+	+	±	±	+	+						
2 weeks	+	+	-	+	±	-	+	+	+	+	+	+	+	+	+
1 month	+	+	-	+	±	-				+	+	+	+	+	+
2 months	±	-	-	+	-	-				+	±	+			
3 months				±			+	+	+	+	+	+	+	+	+
6 months				-			±	-	-	+	+	±	+	+	+
9 months				+	-	-	±	±	-						
12 months				-						+	+	±	+	+	+
18 months				-						+	+	+			
Key:															
+ completely clear when assessed in shadow position ± one or more particles visible - several particles visible on sedimentation															
Activity IU/ml															
T_0				-			-			1410			1368		
1 week				1040	1030	1020									
2 weeks				980	940	700							1296	1230	1164
1 month				1040	1010	940				1130	960	1040	1248	1152	1080
2 months				1000	1000	940	6400	6400	6000	1300	1160	940			
3 months				1040	990	910	5040	5400	5400	1320	1296	1128	1188	1080	912
6 months							6600	6060	4440	1512	1248	360	1440	1044	486
9 months															
12 months										1296	1128	264	1212	972	392
18 months										1272	876	252			

Claims

1. Stable aqueous solution of antibodies, characterized in that it also contains a combination of polyoxypropylene-polyoxyethylene block polymer and phospholipid.

2. Aqueous solution according to claim 1, characterized in that it contains a polyoxypropylene-polyoxyethylene block polymer having a molecular weight between 950 and 4,000 daltons.

3. Aqueous solution according to claim 1 or 2, characterized in that it contains a polyoxypropylene-polyoxyethylene block polymer in which the polyoxyethylene content is at most 80%.

4. Aqueous solution according to claim 3, characterized in that it contains a polyoxypropylene-polyoxyethylene block polymer in which the polyoxyethylene content is at least 50%.

5. Aqueous solution according to claims 1-4, characterized in that it contains lecithin as phospholipid.

6. Aqueous solution according to claims 1-5, characterized in that it contains 0.01-5% polyoxypropylene-polyoxyethylene block polymer.

7. Aqueous solution according to claims 1-6, characterized in that it contains 0.0001-1% phospholipid.

8. Aqueous solution according to claims 1-7, characterized in that it contains 0.001-1 mg of antibody/ml.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 20 2555

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	BIOLOGICAL ABSTRACTS, vol. 82, 1986, abstract no. 37564 Philadelphia, PA, US; S. BENITA et al.: "Physostigmine emulsion; A new injectable controlled release delivery system" & INT J PHARM (AMST) 30(1): 47-56 1986 * Abstract * ---	1-8	A 61 K 47/00 A 61 K 39/395
Y	EP-A-0 085 747 (SCHWEIZERISCHES SERUM- UND IMPFINSTITUT UND INSTITUT ZUR ERFORSCHUNG DER INFEKTIONSKRANKHEITEN) * Page 12, line 28 - page 13, line 7; claims 1-4 * ---	1-8	
Y	EP-A-0 095 751 (EISAI & CO.) * Page 7, lines 7-11; claims 1-10 * ---	1-8	
Y	EP-A-0 231 039 (DE STAAT DER NEDERLANDEN) * Claims 1-14 * -----	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23-12-1988	BERTE M.J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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